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Effects of atrial natriuretic peptide on distal tubule function in humans

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Effects of atrial natriuretic peptide on distal tubule function in humans. To characterize the actions of atrial natriuretic peptide (ANP) in the human distal nephron, we studied interactions between ANP (0.02 $\mu g/kg \cdot min$ i.v.) and acutely administered substances acting in the distal nephron, that is, amiloride and aldosterone, in six healthy humans during maximal water diuresis. ANP increased NaCl excretion, fractional lithium excretion (FE_{Li}) and decreased diluting segment reabsorption estimated from free water clearance. Amiloride increased natriuresis, had no effect on FE_{Li}, but decreased diluting segment reabsorption. Aldosterone had the opposite effect. When infused in addition to amiloride, ANP still increased NaCl excretion, the changes in sodium handling parameters being comparable to those seen after ANP alone. Amiloride did not increase the further natriuretic response to ANP. These findings suggest that ANP increases distal sodium delivery, and decreases sodium reabsorption in distal segments by a mechanism also sensitive to amiloride. ANP abolished much of the antinatriuretic effect of aldosterone, which may also be explained by assuming a partial overlap of the target segments of ANP and aldosterone in the distal nephron. Remarkably, in neither of these experiments was the natriuresis after ANP accompanied by a kaliuresis, for which the explanation remains obscure.

The natriuretic mechanism of ANP probably involves an increase in filtration rate and distal sodium delivery [1, 2]. At the same time, ANP inhibits sodium reabsorption in the collecting ducts; without this effect, the increased distal delivery would cause less natriuresis [3, 4]. In direct studies sodium reabsorption in the rat inner medullary collecting duct (IMCD) was shown to be inhibited [3–5]. The presence in IMCD cells of ANP-specific receptors [6] which respond to ANP with cGMP accumulation [7] and with inhibition of oxygen consumption [8] is in agreement with an action of ANP on sodium transport in this segment. Lately, ANP was also shown to stimulate cGMP production [9] and to decrease sodium reabsorption [10] in the rat cortical collecting duct. In addition, ANP impairs water reabsorption in the rabbit cortical collecting duct [11].

Data on effects of ANP in the human distal nephron (defined here as the terminal nephron starting with the late distal tubule) are not available. Such information may be obtained by combined administration of ANP and substances of which the actions in this segment are known. Amiloride, for instance, decreases sodium reabsorption by inhibiting apical sodium channels in the distal convoluted tubule, perhaps mainly in its late portion [12], and the collecting tubules [13, 14]. Since the natriuretic action of ANP in the collecting tubules is probably mediated by inhibition of amiloride-sensitive sodium entry [15], pretreatment with amiloride may eliminate any distal action of ANP. In this setting the natriuretic effect of ANP would be minimal if its distal action is the main cause of the natriuresis. On the other hand, an enhanced natriuretic effect might result if the main site of action of ANP is upstream from the distal nephron.

Another approach is to alter aldosterone activity. The antinatriuretic action of aldosterone is probably localized mainly in the late distal tubule and cortical collecting tubule [16–18]. Consequently, acute aldosterone administration will diminish sodium delivery to the IMCD. The natriuretic effect of ANP would be greatly curtailed if this were its main site of action. In contrast, in view of the possible action of ANP in the cortical collecting tubule [10], ANP may directly interfere with the antinatriuretic effect of aldosterone. Thus, to further define the effects of ANP in the human distal nephron in vivo we studied in healthy humans the natriuretic response to ANP after pretreatment with either amiloride or aldosterone.

Methods

Studies were carried out in six healthy volunteers (3 males, 3 females), age range 21 to 28 years. Informed consent was obtained and the study was approved by the Hospital Ethical Committee for Studies in Humans.

The subjects took a diet containing 100 mmol Na and 100 mmol K, as outpatients, for eight days. Clearance studies were performed on day 5 and day 8. After an interval of 14 days the diet was restarted, and an additional clearance study was performed on day 5.

The diets were provided by the metabolic ward. Here the subjects returned daily for weighing and to deliver 24-hour urine collections. Lithium carbonate (400 mg) was taken at 10 p.m. on the eve of each clearance study. After a light standardized breakfast (containing 15 mmol Na and 15 mmol K) at 7 a.m., the subjects took the supine position. At 10 a.m. the subjects drank a water load of 20 ml/kg, and additional water equivalent to urine output was supplied for the remainder of the clearance study. A constant infusion of inulin (to estimate glomerular

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filtration rate, GFR) into a lower arm vein was started, preceded by a priming dose. After at least one hour of equilibration, and when urine osmolality had reached a minimal value, three, spontaneously-voided 20-minute urine collections were made. Then a 90 minute i.v. ANP infusion (0.02 μg/kg · min) was started, preceded by a bolus injection of 25 μg (human ANP 99-126, Bissendorf Peptide GmbH, Hannover, FRG). During the infusion period three additional, 30-minute urine collections were made, and the clearance studies were generally finished around 2 p.m. Blood samples for clearance calculations were taken halfway through each collection period via an intravenous canula placed on the lower arm, contralateral to the infusion arm. Blood for plasma renin activity (PRA), aldosterone and ANP was taken 10 minutes before and 75 minutes after the start of the ANP infusion. Blood pressure was recorded with an automatic oscillometer device (Omega 1000, Invivo Res. Lab. Inc., Oklahoma, USA) at five-minute intervals.

Each subject underwent three clearance studies. One was a control study, performed as outlined above. The other two were done during administration of either amiloride or aldosterone. These drugs were administered only on the days of the clearance studies. Amiloride (MSD, Rahway New York, USA) was taken orally in one dose (15 mg) at 8 a.m.; aldosterone (Ciba Geigy GmbH, Wehr, Switzerland) was given i.v. as a 1 mg bolus, followed by 0.5 mg/hour throughout the clearance study. These three clearance studies were performed in a randomized order.

Urine and blood samples were analyzed for osmolality (freezing point depression), sodium and potassium (flame photometry), chloride (technicon RA-autoanalyzer, Tarrytown, New York, USA), lithium (Perkin Elmer 3030 Atomic Absorption Spectrophotometer, Norwalk, Connecticut, USA), and inulin. Inulin was hydrolyzed to fructose and then determined photometrically with indolacetic acid [19]. PRA and plasma aldosterone [20] were determined by radioimmunoassay. ANP was extracted from 2.5 ml plasma by reversed phase chromatography using Baker butyl-silane wide pore extraction columns (Philipsburg, New Jersey, USA), followed by elution with methanol/trifluoro-acetic acid 99:1 vol/vol (recovery of ANP 62%). After evaporation the extract was dissolved in RIA buffer. Aliquots were used for determination by radioimmunoassay using antibody of Peninsula Laboratories (Merseyside, UK) according to the manufacturer's instructions (coefficients of variation inter-assay 17%, intra-assay 11%, ED_{50} 8 fmol, lower limit of sensitivity 0.5 fmol). Values were corrected for percent recovery.

Calculations, statistical analysis

Mean arterial blood pressure was calculated as the sum of one third of the systolic pressure plus two thirds of the diastolic pressure. Clearances were calculated according to standard formula. Free water clearance (C_{H_2O}) during maximal water diuresis was taken as an index of sodium reabsorption in the diluting segment, that is, distal to the point of isotonicity in the medullary ascending limb of Henle's loop. Changes in fractional C_{H_2O} augmented by fractional chloride clearance, written as $[(C_{H_2O} + C_{Cl})/C_{inulin}]$, were taken as an index of changes in fractional solute delivery to the diluting segment, and changes in the equation $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$ as an index of changes in diluting segment reabsorption. The validity of these equations has been discussed by others [21].

Table 1. Steady state data before the clearance studies

	Control	Amiloride	Aldosterone
Body weight *kg*	71.6 ± 4.4	71.4 ± 4.4	71.7 ± 4.3
Urine sodium *mmol/24 hr*	101 ± 13	94 ± 11	94 ± 6
Urine potassium *mmol/24 hr*	79 ± 6	72 ± 9	66 ± 6

Values are given as the mean ± standard error. PRA and aldosterone data were evaluated after logarithmic transformation. Statistical analysis was performed using two-way analysis of variance of a randomized block design. If the variance ratio's obtained by this method reached statistical significance, the differences between the means were analyzed at 5% and 1% significance level by the Least Significance Difference test.

Results

There were no significant differences in body weight, mean arterial pressure and 24 hour excretion of sodium and potassium prior to the clearance studies (Table 1).

Effects of ANP

Figure 1 shows that there was a gradual rise in sodium excretion throughout the ANP infusion in each of the three clearance studies. The largest changes were present in the third collection period. For subsequent presentation and evaluation of the data (Table 2 and Fig. 2) this collection period was compared to baseline, taken as the mean of the final two collection periods prior to ANP infusion.

In the control study absolute and fractional sodium chloride excretion increased to approximately twofold after ANP (Table 2). There was only a slight rise in GFR which did not reach significance. Potassium excretion decreased (Fig. 1, Table 2). Fractional excretion of lithium increased. No significant change was found in maximal urine flow, free water clearance and diluting segment delivery term, although each of these items tended to increase. There was a rise in minimal urine osmolality and a decrease in diluting segment reabsorption, as assessed by the term $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$. The infusion of ANP was accompanied by an about sevenfold rise in plasma immunoreactive ANP, and a decrease in plasma renin activity and aldosterone (Table 3). There was no consistent change in blood pressure.

Effects of amiloride

Amiloride increased sodium excretion about threefold compared to control (Fig. 2). There also was marked potassium retention. No significant change was found in GFR, maximal urine flow, fractional lithium excretion and the diluting segment delivery term, but minimal urine osmolality increased and the diluting segment reabsorption term decreased compared to control. When infused in this setting ANP had an additional natriuretic effect, and sodium excretion rate was higher than in the corresponding phase of the control study. The absolute increment in sodium excretion in the third 30-minute period of ANP was 106 ± 54 μmol/min, somewhat less than observed in

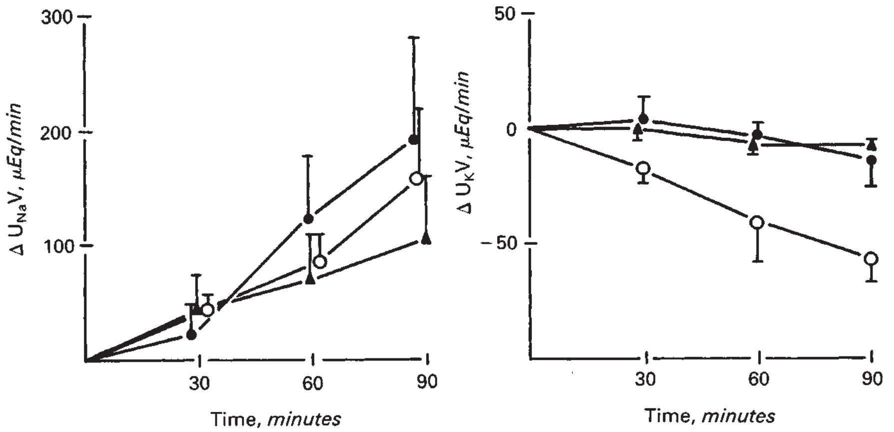

Fig. 1. *Changes from baseline (0 min) in urine sodium excretion (Δ UN_aV) and potassium excretion (Δ U_KV) during 90 minutes of ANP infusion in control conditions (—○—), and after pretreatment with amiloride (—▲—) or aldosterone (—●—).* The rise in sodium excretion was always significant at 90 minutes of infusion ($P < 0.01$, not indicated in the figure); urine potassium excretion fell significantly in the control study ($P < 0.05$) but not in the other two studies.

Table 2. Clearance data

	Control		Amiloride		Aldosterone	
	Baseline	ANP	Baseline	ANP	Baseline	ANP
C_{inulin} *ml/min*	119 ± 9	127 ± 5	115 ± 10	124 ± 10	113 ± 8	132 ± 10[a]
Urine sodium *μmol/min*	124 ± 27	281 ± 83[b]	361 ± 48[d]	467 ± 83[b,d]	24 ± 7[d]	215 ± 96[b]
Urine potassium *μmol/min*	133 ± 13	81 ± 8[b]	54 ± 8[d]	47 ± 8[c]	118 ± 14	107 ± 15[c]
Urine chloride *μmol/min*	116 ± 25	200 ± 73[a]	185 ± 24[c]	293 ± 55[b,d]	48 ± 10[c]	202 ± 88[b]
Urine osmolality *mOsmol/kg*	61 ± 3	67 ± 5[b]	79 ± 3[d]	79 ± 4[d]	60 ± 5	62 ± 5[d]
Urine flow *ml/min*	13.3 ± 1.4	14.5 ± 2.1	14.3 ± 1.7	16.4 ± 2.0	11.5 ± 1.3	16.8 ± 3.1[b]
FE_{Na} *%*	0.77 ± 0.16	1.60 ± 0.44[b]	2.34 ± 0.23	2.70 ± 0.38[a]	0.15 ± 0.04	1.09 ± 0.45[a]
FE_{Li} *%*	25.7 ± 2.0	28.1 ± 2.4[a]	26.2 ± 1.7	29.1 ± 1.4[a]	26.6 ± 2.3	31.7 ± 2.5[b]
C_{H_2O}/C_{inulin} *%*	8.7 ± 0.7	9.1 ± 0.9	8.9 ± 0.6	9.1 ± 0.7	8.0 ± 0.8	9.6 ± 1.2[b]
$(C_{H_2O} + C_{Cl})/(C_{inulin})$ *%*	9.7 ± 0.8	10.8 ± 1.3	10.5 ± 0.8	11.4 ± 1.0	8.5 ± 0.8	11.0 ± 1.7[b]
$(C_{H_2O})/(C_{H_2O} + C_{Cl})$ *%*	90.2 ± 1.4	85.6 ± 2.9[b]	84.5 ± 1.4[d]	80.6 ± 1.9[b,d]	95.2 ± 0.6[d]	89.2 ± 3.1[b,d]

Data before ("baseline") and during the 3rd half hour of ANP infusion ("ANP") in control conditions, and after pretreatment with amiloride or aldosterone. Abbreviations are: C, clearance; FE, fractional excretion; ANP, atrial natriuretic peptide.

Significant differences between baseline period and ANP infusion are denoted by [a] $P < 0.05$, and [b] $P < 0.01$. Significant differences between control values and corresponding values in either of the other two studies are indicated by [c] $P < 0.05$, and [d] $P < 0.01$.

the control study (157 ± 59 μmol/min), but this difference was not significant. Again, the natriuresis was accompanied by an increase in the fractional lithium excretion and a fall in the term $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$, although there was no further change in minimal urine osmolality. Potassium excretion was unaltered (−7.4 μmol/min, NS), in contrast with the control study (−52.3 μmol/min, $P < 0.05$). The changes in hormonal parameters were comparable to the changes observed in the control study.

Effects of aldosterone

Aldosterone increased plasma aldosterone concentration substantially (Table 3). Sodium excretion decreased markedly, whereas potassium excretion did not change. While there was no significant influence of aldosterone on fractinal lithium excretion, maximal urine flow and free water clearance tended to be lower than in control conditions. The diluting segment reabsorption term was now elevated. In this situation too, ANP infusion increased sodium excretion, the absolute rise (191 ± 90 μmol/min in the third half hour of ANP) again being not significantly different from the increase seen in the control study (Fig. 1). Different from the other two studies, the rise in GFR and urine flow during ANP infusion now reached significance. There was a marked rise in fractional lithium excretion as well. The diluting segment reabsorption term again decreased, but remained relatively high as compared to the control study. The changes in plasma renin activity and ANP were similar compared to the control study. There was a tendency for plasma potassium to be lower during aldosterone infusion. Again, potassium excretion did not change (−12 μmol/min, NS).

Discussion

The main finding of the present study in humans is that ANP retained its natriuretic effect after a high dosage of aldosterone, even though the latter reduced pre-ANP sodium excretion markedly. The absolute increase in sodium excretion after ANP was also not influenced by pretreatment with amiloride, although this drug had increased pre-ANP sodium excretion.

The effects of ANP infusion on renal function were comparable to those seen earlier [22, 23]. In short, the data are compatible with increased filtered load and decreased fractional reabsorption of sodium. The fractional excretion of lithium, a rough index of end-proximal sodium delivery [24], increased, while the term $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$, representing diluting segment reabsorption [21], decreased. The term $[(C_{H_2O} + C_{Cl})/(C_{inulin})]$ and maximal urine flow tended to increase, although these changes were not as marked as seen in earlier studies using similar dosage of ANP [22, 23]. Despite method-

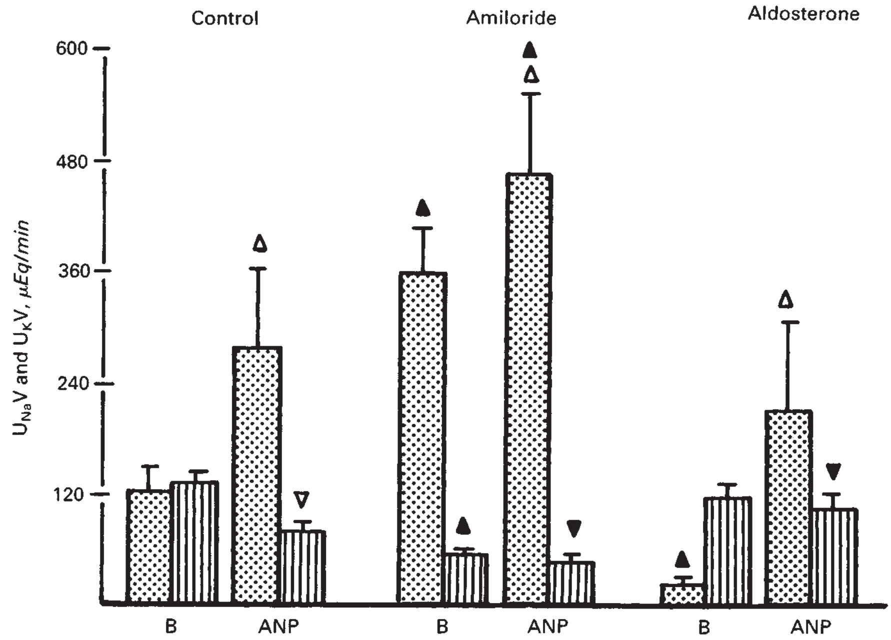

Fig. 2. *Urine sodium excretion (UN_aV, ▨) and potassium excretion (U_KV, ▥) at baseline (B) and in the 3rd half hour of ANP infusion (ANP) during control conditions, and after pretreatment with amiloride or aldosterone.* Significant differences between baseline and ANP infusion are denoted by ▽, $P < 0.05$, and △, $P < 0.01$. Significant differences between control value and corresponding values of the other two studies are denoted by ▼, $P < 0.05$, and ▲, $P < 0.01$.

Table 3. Effects of ANP on humoral factors, plasma potassium and blood pressure

	Control		Amiloride		Aldosterone	
	Baseline	ANP	Baseline	ANP	Baseline	ANP
PRA *fmol AI/l s*	250 ± 63	167 ± 45[b]	198 ± 66	159 ± 40[a]	282 ± 83	217 ± 74
Aldosterone *pmol/liter*	311 ± 90	172 ± 28[b]	265 ± 74	130 ± 38[b c]	28000 ± 5146[d]	28000 ± 4137[d]
ANP *pmol/liter*	23 ± 5	168 ± 26[b]	20 ± 4	203 ± 39[b]	21 ± 3.0	174 ± 31[b]
Plasma K *mmol/liter*	3.83 ± 0.11	3.70 ± 0.15	3.93 ± 0.16	3.87 ± 0.08	3.48 ± 0.11	3.47 ± 0.10
MAP *mm Hg*	89 ± 2	90 ± 2	87 ± 2	84 ± 2	88 ± 3	87 ± 3

Data before ("baseline") and during the 3rd half hour of ANP infusion ("ANP") in control conditions, and after pretreatment with amiloride or aldosterone. Abbreviations are: PRA, plasma renin activity; ANP, atrial natriuretic peptide; MAP, mean arterial pressure.

Significant differences between baseline period and ANP infusion are denoted by [a] $P < 0.05$, and [b] $P < 0.01$. Significant differences between control values and corresponding values in either of the other two studies are indicated by [c] $P < 0.05$, and [d] $P < 0.01$.

ological uncertainties it seems fair to assume that ANP suppressed fractional sodium reabsorption in both proximal and diluting segments. An effect in the proximal tubules may be hemodynamically mediated, but also a direct one, as suggested in recent studies [25, 26]. By definition, the diluting segment comprises the entire nephron lying distally from halfway the medullary thick ascending limb of Henle's loop [21, 27]. We have tried to further characterize the distal action of ANP by infusing ANP in combination with amiloride and aldosterone, which act mainly in the distal nephron. These drugs had no effect on the initial plasma ANP concentration, and infusion of ANP consistently increased plasma ANP by about eightfold.

Amiloride, by itself, increased sodium excretion and decreased potassium excretion. Presumably, amiloride caused natriuresis by inhibiting sodium entry through apical sodium channels throughout the distal nephron, but, due to the limited dosage, had no proximal effect [13, 28]. This accords with the clearance data, showing no change in lithium clearance and distal delivery term, but a marked rise in minimal urine osmolality and the term $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$. Addition of ANP increased sodium excretion further, the rise being somewhat but not significantly less than seen in the control study. This finding confirms recent results in rabbits [29], and indicates that ANP can increase natriuresis by a mechanism insensitive to amiloride.

One option is that ANP decreases proximal tubule sodium reabsorption by a mechanism not influenced by the presently used dosage of amiloride. Indeed, ANP increased lithium clearance also when given on top of amiloride.

Our objective, however, was to find out whether pretreatment with amiloride would interfere with the action of ANP on the distal nephron. To evaluate this, we have to assume that ANP infusion caused a comparable decrease in proximal reabsorption and, probably, increase in delivery to the distal nephron in the studies with and without amiloride. A different magnitude of the (further) increase in sodium excretion following ANP infusion would then be expected if the extent to which sodium reabsorption in the distal nephron is suppressed were different. Thus, if the actions of amiloride and ANP to suppress sodium reabsorption in the distal nephron are independent, one would expect a much larger acute increase in sodium excretion when ANP is given on top of amiloride than when ANP is infused alone. This was evidently not the case, suggesting that an effect of ANP on the distal nephron did not come to expression during amiloride. This idea, based on indirect evidence, is in accordance with more direct data in rabbits, in which amiloride was shown to block sodium channels in the IMCD which were probably also ANP sensitive [15]. On the other hand, amiloride has been shown to increase ANP binding to adrenal ANP receptors [30], and it remains difficult to say how, but also where in the distal nephron amiloride and ANP may interact.

Administration of aldosterone caused a strong retention of sodium chloride, and a rise in the term $[(C_{H_2O})/(C_{H_2O} + C_{Cl})]$, compatible with increased sodium reabsorption in the collecting ducts. Potassium excretion remained nonetheless unchanged, an at first sight unexpected finding also reported by others [31]. Absence of acute kaliuresis probably has multiple causes, one being the acute fall in plasma potassium [32] as also found in the present study. In this setting the increase in natriuresis caused by ANP was not at all blunted. In fact, ANP cancelled much of the antinatriuretic effect of aldosterone. There are two possible explanations for this finding. First, ANP infusion may have elevated sodium delivery to the main sites of aldosterone action so much that a part of it escaped reabsorption. This must be weighed against the fact that the aldosterone infusion appeared able to cause virtually complete reabsorption of distal delivery prior to ANP. However, the rise in GFR and maximal urine flow after ANP were exceptionally large in this experiment. Although the reason for this effect is unclear, it is compatible with an unusually large increase in distal delivery.

The second option is that ANP and aldosterone act at least partly in overlapping target segments in the distal nephron, where ANP now inhibited the antinatriuretic effect of aldosterone. Conceivably, aldosterone not only stimulated sodium reabsorption along the collecting tubules, but, consequently, also water back diffusion, even although the studies were performed during water loading. This could explain the relatively low urine flow during aldosterone, a change also cancelled by ANP. This possibility of partially overlapping target nephron segments is in keeping wiht the findings during amiloride.

Since the main site of action of aldosterone is the late distal tubule and cortical collecting tubule [16–18], an action of ANP would be suspected here. This is in agreement with recent findings that ANP increases cGMP accumulation [9] and lowers sodium reabsorption [10] in isolated perfused rat cortical collecting ducts. The rats in the later study were pretreated with deoxycorticosterone. Therefore it was held likely that aldosterone-sensitive sodium transport and thus also the responsiveness to ANP may have been enhanced in these tubules [10]. This is in accordance with our in vivo finding that the natriuretic effect of ANP is greatly enhanced in humans escaped from the sodium retaining effect of fludrocortisone [22]. Whether ANP decreases sodium reabsorption in the aldosterone-sensitive collecting tubules by inhibition of conductive sodium transport, as in the case in the IMCD [15], or by another mechanism, remains to be elucidated.

Alternatively, aldosterone and ANP may interact in the IMCD. This possibility cannot be ruled out, although the major sodium retaining effect of aldosterone is prior to this segment. Two micropuncture studies in rats showed that adrenalectomy without [33] or with [34] corticosteroid suppletion diminished sodium reabsorption in this segment. This would indicate a controlling role of aldosterone also in the physiological range, and not only in pharmacological amounts as given in the present study. It has been reported lately that desoxycorticosterone may increase the reabsorption of sodium chloride in the IMCD of the rat by diminishing its passive backleak [35] and by stimulating Na^+, K^+-ATPase [36]. Whether this is quantitatively important for the antinatriuretic effect of mineralocorticoid is unknown, but it is conceivable that ANP could neutralize this effect by preventing sodium chloride absorption through the IMCD apical membrane [15, 35].

As in previous studies [22, 23], the natriuresis induced by ANP was not associated with a kaliuresis. In fact, in the control study a fall in potassium excretion was noticed during the infusion of ANP. A recent study in humans showed the same fall in potassium excretion during low dose ANP infusion, but a similar change in a time control study [37]. Anyway, it is remarkable that the natriuresis after ANP was never accompanied by a rise in potassium excretion. Since the role of the IMCD in potassium secretion is probably limited [18, 38], absence of a consistent effect of ANP on potassium excretion would be reconcilable with limitation of its distal effects to the IMCD. However, this cannot explain why no larger kaliuresis occurred when ANP was administered during infusion of aldosterone. Again, the low plasma potassium concentration may be have impaired kaliuresis [32]. Also, since antidiuretic hormone seems essential as regulating or permissive factor in potassium secretion in various parts of the distal nephron [39, 40], the water-loading conditions of our study may have impaired a rise in potassium excretion.

In summary, the results of these clearance studies in humans accord with the notion that ANP causes natriuresis by increasing delivery to the distal nephron and by suppressing reabsorption in a segment of the distal nephron. The latter may very well concern the IMCD. However, in accordance with recent animal data, the present results also speak for inhibition of aldosterone-sensitive sodium reabsorption, thus for an effect in the cortical collecting tubules.

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